Protocol Title: **A**lvelestat (MPH966) for the **T**reatment of **AL**pha-1 **ANT**itrypsin Deficiency (ATALANTa)

A Phase 2, multicenter, double-blind, randomized, placebo-controlled study to evaluate efficacy, safety, and tolerability of alvelestat (MPH966) in alpha-1 antitrypsin deficiency

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1. Synopsis

Protocol Title: Alvelestat (MPH966) for the Treatment of ALpha-1 ANTitrypsin Deficiency (ATALANTa)

Rationale:

Alpha-1 antitrypsin deficiency (AATD) is the most common genetic cause of chronic obstructive pulmonary disease (COPD) and early-onset emphysema. AATD is characterized by low AAT levels; leading to excessive neutrophil elastase (NE) mediated lung destruction. Current treatment requires the periodic infusion of pooled AAT derived from human plasma, but this therapeutic approach (termed augmentation) does not definitively slow the rate of lung function decline and is very expensive. In addition, it is not clear that the currently recommended dose for augmentation fully controls lung inflammation and destruction. Alvelestat (MPH966, formerly AZD9668) is a potent, selective, and reversible, oral inhibitor of human NE. Suppression of NE is expected to reduce lung damage and may slow disease progression. This study is to establish proof of clinical concept by investigating the mechanistic effect and safety of alvelestat (MPH966) in patients with AATD.

Overall Design:

This is a Phase 2, multicenter, double-blind, randomized (1:1), placebo-controlled, 12-week, proof-of-concept study to evaluate the safety and tolerability as well as the mechanistic effect of oral administration of alvelestat (MPH966) in subjects with confirmed AATD defined as Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low (serum AAT level <11 μM or <57.2 mg/dL) or functionally impaired AAT including “F” or “I” mutations.

Objectives and Endpoints:

Objective	Endpoint
Primary	
• To evaluate the effect of alvelestat (MPH966) administered twice daily (bid) for 12 weeks on blood markers of neutrophil elastase activity	• Within-individual % change from baseline in plasma desmosine/isodesmosine at end of treatment with MPH966
• To evaluate the safety and tolerability of alvelestat (MPH966) administered twice daily (bid) for 12 weeks	• Numbers and % of subjects who experience at least 1 treatment-emergent adverse event • Adverse events of special interest (liver function abnormalities, corrected QT interval/cardiac, infections, and neutropenia)
Secondary	
• To evaluate the effect of alvelestat (MPH966) on other blood pharmacodynamic markers of neutrophil activation and elastase activity	• Change from baseline in plasma desmosine/isodesmosine at end of treatment compared to placebo • Change from baseline in blood Aα-Val^{360}, NE, EL-NE, Proteinase 3, and Cathepsin B at end of treatment compared to placebo
• To evaluate the effect of alvelestat (MPH966) on blood biomarkers of lung tissue degradation	• Change from baseline in blood EL-CG, EL-P3, PGP, C6M, C1M, and PRO-C6, at end of treatment compared to placebo

Objective	Endpoint
• To evaluate the effect of alvelestat (MPH966) on biomarkers of inflammation in blood	• Change from baseline in blood high sensitivity C reactive protein, interleukin [IL]-6, IL-8, IL-1β, RANTES, fibrinogen, MMPs, and MPO at end of treatment compared to placebo
• To evaluate the effect of alvelestat (MPH966) on neutrophil activation, elastase, and inflammatory activity in lung	• Change from baseline in desmosine/isodesmosine, NE, NE activity, Aα-Val^{360}, EL-NE, Proteinase 3, and Cathepsin B; inflammatory biomarkers (IL-6, IL-8, IL-1β, LTB4, RANTES, PGP) at end of treatment in bronchoalveolar lavage (BAL) compared to placebo • Change from baseline in desmosine/isodesmosine, NE, NE activity, Aα-Val^{360}, EL-NE, Proteinase 3, and Cathepsin B, and inflammatory biomarkers (IL-6, IL-8, IL-1β, LTB4, RANTES, PGP) at end of treatment in induced sputum compared to placebo
Exploratory	
• To evaluate the effect of alvelestat (MPH966) on pulmonary function	• Change from baseline in pre-and post-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC (total and percent predicted), and maximal mid-expiratory flow at end of treatment
• To evaluate the effect of alvelestat (MPH966) on respiratory symptoms, breathlessness, and health status	• Change from baseline in St. George's Respiratory Questionnaire (SGRQ), COPD Assessment Test (CAT), Modified Medical Research Council Questionnaire (MMRC), San Diego Breath Questionnaire (SOBQ) and daily symptom scores
• To evaluate PK efficacy relationships in AATD	• PK/pharmacodynamic (PD) relationship with efficacy biomarkers in blood and sputum

2. Schedule of Activities (SOA)

Visit #	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Visit Description	Pre-bronch/ Screen	Baseline bronch	Randomize	Safety F/U	Safety F/U	4 week F/U	Safety F/U	8 week F/U	Safety F/U and Pre-bronch	End of study bronch	Study End	Washout End
Week (from randomization)	-4 to 0	-1	0	+1	+2	+4	+6	+8	+10	+11	+12	+16
Randomization			X									
Baseline history	X											
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
Interim history and adverse event recording		X	X	X	X	X	X	X	X	X	X	X
Blood draw (routine/safety)	X			X	X	X	X	X	X		X	X
Blood draw (biomarkers)	X		X			X		X			X	X
Blood (PK)			X@	X	X	X		X	X		X	
Pregnancy test	X		X	X	X	X	X	X	X		X	X
ECG	X		X@	X	X	X		X	X		X	X
Sputum biomarkers	X										X	
Spiro- pre and post BD			X			X					X	X
Spiro – post BD	X								X			
PROs#			X			X					X	X
Bronchoscopy		X								X		
Phone contact		X (day after)								X (day after)		
Electronic diary	X		X	X	X	X	X	X	X	X	X	X

#Patient reported outcomes to include Modified Medical Research Council questionnaire (MMRC), COPD assessment test (CAT), St George Respiratory Questionnaire (SGRQ), San Diego Shortness of Breath Questionnaire; @ pre-dose and between 1 and 2 hours post-dose

3. Introduction

3.1. Background

COPD affects nearly 24 million individuals and is the fourth leading cause of death in the United States[1-4]. COPD is a heterogeneous disease characterized by progressive airflow obstruction, excessive inflammation, and protease/anti-protease imbalance [5,6]. AATD is the most common genetic cause of COPD and emphysema, accounting for 1-4% of cases of COPD in the United States and affecting between 70,000-100,000 individuals [7-9]. AAT is encoded by the SERPINA1 gene and mutations in SERPINA1 cause absence or misfolding of the AAT protein, resulting in severely reduced plasma and lung levels of AAT. The most common variants conveying increased risk for COPD associated with AATD are the Pi*ZZ and Pi*(null) (null) phenotypes, accounting for very low or absent AAT respectively. In contrast to the general COPD population, individuals with AATD have early-onset severe emphysema, a more rapid decline in lung function, and an increased risk for liver dysfunction [10]. The disease often affects people in the fourth and fifth decades of life, leading to substantial loss of productivity and societal contributions[7]. Current therapies for AATD include the use of bronchodilators, inhaled corticosteroids, and intravenous AAT augmentation using pooled, human plasma derived AAT. AAT augmentation is indicated for individuals with emphysema and AATD, including individuals with the Pi*ZZ, Pi*SZ, Pi*Z (null), or Pi*(null) (null) mutations. AAT augmentation has been shown to slow the loss of lung parenchyma as measured by CT lung density, but unfortunately does not definitively affect lung function decline, respiratory symptoms, exacerbations, or survival[9,11]. Further, AAT augmentation is very expensive with direct annual costs exceeding $100,000 per patient including the costs associated with technical expertise required for repeated intravenous infusion. Recent studies also suggest that the currently recommended dose of augmentation does not adequately control NE activity, lung inflammation and consequent lung destruction pointing to the need for additional NE inhibition to improve outcomes[12].

The mechanisms by which AATD leads to COPD and emphysema are based primarily on the paradigm of protease-antiprotease imbalance [7,8,13]. AAT is a member of the serine protease inhibitor superfamily of proteins which play a role in inactivating NE and other proteases[14,15]. AAT reacts with NE much more readily than other proteases, including proteinase 3 (PR3), cathepsin G (CatG), and trypsin, and in the lung plays a major role in mitigating against NE-mediated elastolysis. In AATD, individuals not only have quantitatively less AAT, but the enzyme is less effective in inhibiting NE[16]. NE is a protease stored in the azurophilic granules of neutrophils and causes more robust proteolysis and connective tissue degradation compared to other proteases, including CatG and PR3, contained in the same azurophilic granules [17]. Under normal circumstances, NE is regulated by AAT, however, in conditions like COPD, and in particular AATD, the extracellular free enzyme NE concentration exceeds the buffering capacity of AAT [7,15]. Thus, NE causes direct lung tissue destruction and initiates a cascade resulting in pulmonary inflammation, mucus overproduction, and further damage to lung tissue and extracellular matrix[18,19].

Elastin is responsible for the elasticity of the lungs and is the substrate for NE[20-22]. Elastin is a highly insoluble protein formed by cross-linking tropoelastin with desmosine and isodesmosine (collectively referred to as DES). Elastin degradation generates several elastin fragments, including DES[20]. As these molecules are found exclusively in mature elastin in humans, they are useful biomarkers for measuring lung elastin degradation and have been measured in lung tissue, sputum, bronchoalveolar lavage (BAL), urine, and blood in patients with AATD[20,23-25]. DES as well as other NE-specific markers of elastin or collagen turnover (EL-NE) are associated with CT emphysema, lung function, and with other markers of inflammation in COPD and in AATD [26-30]. Further, AAT augmentation therapy has been demonstrated to reduce DES levels, validating their use as a marker for target modulation in trials targeting NE [31].

MPH966 (previously named AZD9668) is a potent, selective, and orally available neutrophil elastase (NE) inhibitor[32,33]. It has been developed as treatment for pulmonary diseases characterized by increased protease activity and neutrophilic inflammation, including bronchiectasis, cystic fibrosis (CF), and COPD[33-38]. In Phase 2 clinical trials, MPH966 improved lung function in bronchiectasis, reduced markers of elastin degradation in CF, and reduced inflammatory markers in non-AATD patients with COPD. MPH966 was well tolerated in these trials

and no significant safety concerns were raised. However, in the COPD studies, MPH966 failed to achieve the primary efficacy endpoint of lung function improvement, due in part to the heterogeneous nature of population enrolled[33,34]. AATD leads to the only true unique endotype in COPD [19,39] and is caused by a genetic mutation leading to unopposed NE activity which in turn promotes accelerated lung destruction and early-onset emphysema[7,8]. Thus, AATD related COPD is the most ideally suited disease to test anti-NE therapy, and this project will examine the potential benefit of MPH966.

MPH966 has high binding affinity and potently inhibits NE activity with an IC_{50} of 12 nM and K_i of 9.4 nM for human NE[32]. MPH966 abrogated acute lung injury in mice and rats and decreased pulmonary inflammation (neutrophils and IL-1b) in smoking injury models[32]. In chronic smoke exposure models in guinea pigs, MPH966 prevented airspace enlargement and alveolar remodeling. In *ex vivo* experiments, MPH966 inhibited zymosan-stimulated NE activity in human blood, with an IC_{95} at plasma concentrations of 300nM[32]. A detailed description of the chemistry, pharmacology, efficacy, and safety of alvelestat (MPH966) is provided in the Investigator Brochure (Version 12).

To date, 12 clinical trials have been conducted using MPH966 in non-CF bronchiectasis, CF, bronchiolitis obliterans syndrome, and COPD. Although none of these trials achieved their primary endpoint, significant improvements in FEV_1 were observed in non-CF bronchiectasis and improvement in inflammatory markers were observed in CF and in non-AATD related COPD. These trials compared placebo to MPH966 60mg twice per day and plasma MPH966 levels averaged 200nM, suggesting the 60mg dose was at the lower end of the dose range to achieve adequate target engagement. Thus, a higher dose of MPH966 is needed to achieve the IC_{95} (plasma MPH966 concentration of >300nM). In all previous trials, MPH966 was well tolerated with no treatment emergent significant adverse events, ECG or vital sign changes, and no dose related liver function abnormalities were observed.

The scientific premise that NE inhibition ameliorates ongoing elastolysis in AATD will be tested. If MPH966 blocks NE activity in the lungs of AATD patients with PI*ZZ, Pi*SZ, or PI*(Null) (Null) phenotypes, we predict that it will provide the first disease-modifying treatment in AATD and provide a paradigm shifting oral therapeutic strategy. This would transform the care of an orphan disease in a patient population with an unmet medical need. The first stage in the development of alvelestat (MPH966) in AATD-related emphysema is this proof-of-concept trial. The results of this study will determine progression to phase 3 clinical efficacy studies.

3.2. Study Rationale

The aims of this study are to build on the previous alvelestat (MPH966) clinical experience and to confirm mechanistic efficacy through elastase inhibition in the AATD population and investigate the safety and tolerability of alvelestat (MPH966) in the target population. Clinical efficacy endpoint studies in AATD-related lung disease often involve longer-term studies and are therefore inappropriate for decision making at the early stages of clinical development. The availability of biomarkers of elastolysis, which have been investigated in AATD, correlate with lung damage, and show treatment response over periods of 4 to 12 weeks [23,25,31], enables a study design to demonstrate mechanistic effects over an appropriate time period for a proof-of-concept trial. Greater efficacy and clinical benefit is expected by targeting patients with AATD-related lung disease, in which neutrophil elastase is considered the key pathogenic driver, and by using a dose of alvelestat (MPH966) to maintain neutrophil elastase inhibition throughout the 24-hour period.

Biomarkers of inflammation and lung matrix breakdown will be used to provide evidence that the inhibitory effect of alvelestat (MPH966) on neutrophil elastase may deliver potential clinical benefit demonstrated by effects on the pathogenic progresses that drive disease progression. This potential will be tested in patients receiving augmentation, which is the standard of care in many countries, as well as in those not receiving augmentation. These efficacy data in combination with safety and tolerability will be used to make progression decisions on alvelestat (MPH966) development in AATD.

4. Objectives and Endpoints

Objective	Endpoint
Primary	
• To evaluate the effect of alvelestat (MPH966) administered twice daily (bid) for 12 weeks on blood markers of neutrophil elastase activity	• Within-individual % change from baseline in plasma desmosine/isodesmosine at end of treatment with MPH966
• To evaluate the safety and tolerability of alvelestat (MPH966) administered twice daily (bid) for 12 weeks	• Numbers and % of subjects who experience at least 1 treatment-emergent adverse event • Adverse events of special interest (liver function abnormalities, corrected QT interval/cardiac, infections, and neutropenia)
Secondary	
• To evaluate the effect of alvelestat (MPH966) on other blood pharmacodynamic markers of neutrophil activation and elastase activity	• Change from baseline in plasma desmosine/isodesmosine at end of treatment compared to placebo • Change from baseline in blood Aα-Val^{360}, NE, EL-NE, Proteinase 3, and Cathepsin B at end of treatment compared to placebo
• To evaluate the effect of alvelestat (MPH966) on blood biomarkers of lung tissue degradation	• Change from baseline in blood EL-CG, EL-P3, PGP, C6M, C1M, and PRO-C6, at end of treatment compared to placebo
• To evaluate the effect of alvelestat (MPH966) on biomarkers of inflammation in blood	• Change from baseline in blood high sensitivity C reactive protein, interleukin [IL]-6, IL-8, IL-1β, RANTES, fibrinogen, MMPs, and MPO at end of treatment compared to placebo
• To evaluate the effect of alvelestat (MPH966) on neutrophil activation, elastase, and inflammatory activity in lung	• Change from baseline in desmosine/isodesmosine, NE, NE activity, Aα-Val^{360}, EL-NE, Proteinase 3, and Cathepsin B; inflammatory biomarkers (IL-6, IL-8, IL-1β, LTB4, RANTES, PGP) at end of treatment in bronchoalveolar lavage (BAL) compared to placebo • Change from baseline in desmosine/isodesmosine, NE, NE activity, Aα-Val^{360}, EL-NE, Proteinase 3, and Cathepsin B, and inflammatory biomarkers (IL-6, IL-8, IL-1β, LTB4, RANTES, PGP) at end of treatment in induced sputum compared to placebo
Exploratory	

Objective	Endpoint
• To evaluate the effect of alvelestat (MPH966) on pulmonary function	• Change from baseline in pre-and post-bronchodilator forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC (total and percent predicted), and maximal mid-expiratory flow at end of treatment
• To evaluate the effect of alvelestat (MPH966) on respiratory symptoms, breathlessness, and health status	• Change from baseline in St. George's Respiratory Questionnaire (SGRQ), COPD Assessment Test (CAT), Modified Medical Research Council Questionnaire (MMRC), San Diego Breath Questionnaire (SOBQ) and daily symptom scores
• To evaluate PK efficacy relationships in AATD	• PK/pharmacodynamic (PD) relationship with efficacy biomarkers in blood and sputum

5. Study Design

5.1. Overall Design

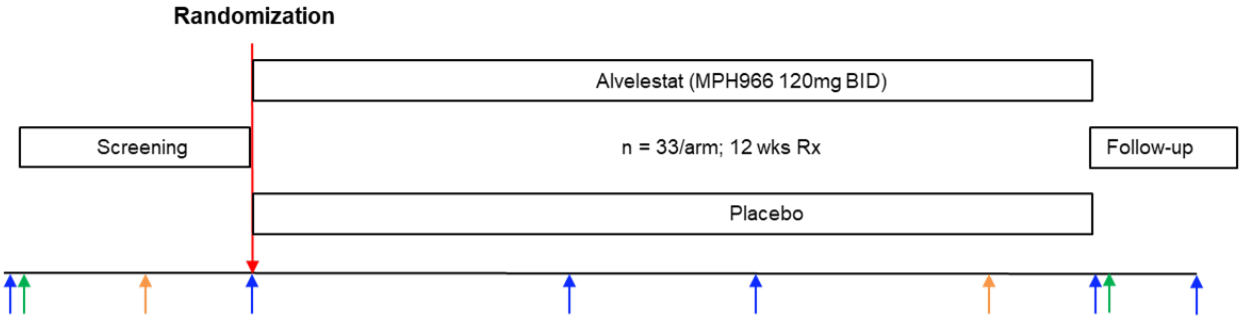
This will be a multicenter, double-blind, randomized, placebo-controlled trial. One dose of alvelestat (MPH966), 120 mg bid, will be tested against placebo. These doses are based on PK and PD modelling of alvelestat (MPH966) inhibition of human neutrophil elastase.

The participants will be those patients with the AATD at risk for emphysema (Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low (serum AAT level <11 µM or <57.2 mg/dL) or functionally impaired AAT including “F” or “I” mutations)

Following a screening period of up to 4 weeks, patients will be dosed with study treatment or placebo for 12 weeks, with a subsequent 4-week follow-up period for safety and determination of the offset of the mechanistic effect of MPH966 on the primary endpoint (desmosine/isodesmosine).

The study is divided into 3 phases: a screening phase, treatment phase, and washout phase as detailed below:

Figure 1: **Study Schematic**



Visit	1	2	3	4	5	6	7	8	9	10	11	12
Week	-4 to 0	-1	0	+1	+2	+4	+6	+8	+10	+11	+12	+16
Description	Screen	Bronch	Randomization	Safety	Safety	4-week interim	Safety	8-week interim	Safety and Pre-bronch	Bronch	Study End	Post-IP Washout

Red arrow = Randomization
Blue arrow = Blood (biomarker) collection
Green arrow = Sputum collection
Orange arrow = BAL collection

- **A screening period** from Week -4 to Day -1. Baseline safety assessments and measurement of DES in blood, sputum, and BAL (in those who consent to bronchoscopy) will occur prior to randomization and treatment allocation.

- **A treatment period** consisting of alvelestat (MPH966) 120mg bid or placebo for 12 weeks. Safety assessments including tests of liver function, ECG, and chemistries will be assessed at the week 1 and week 2 visits and then bi-weekly for the remainder of the 12-week treatment period. Blood will be collected at the Week 4, 8, and 12 visit for biomarker analysis. Sputum will be collected at the week 12 visit. Follow-up BAL will be collected at week 11.

- **A washout period** from Week 12 to Week 16. Blood will be collected for biomarker analysis at week 16.

5.2. Number of Participants

A maximum of 66 participants will be randomized so that approximately 60 evaluable participants complete the study.

5.3. End of Study Definition

The end of the study is defined as the date of all data being entered into the study database and the database being locked and ready for analysis.

5.4. Scientific Rationale for Study Design

The aims of this study are to build on the previous alvelestat (MPH966) clinical experience and to confirm its mechanistic efficacy through elastase inhibition in the AATD population and to investigate the safety and tolerability of alvelestat (MPH966) in the target population.

Clinical efficacy endpoint studies in AATD-related lung disease involve long-term treatment due to the slow rate of structural lung and functional decline and are therefore inappropriate for decision making at the early stages of drug development. The availability of biomarkers of neutrophil elastase activity (such as desmosine/isodesmosine and Aα-Val^{360}), which have been investigated in AATD, correlate with lung damage and show treatment response over periods of 4 to 12 weeks, enables a study design to demonstrate mechanistic effects over an appropriate time period for a proof-of-concept trial[40]. Biomarkers of inflammation and lung matrix breakdown will be used to provide evidence that the inhibitory effect of alvelestat (MPH966) on neutrophil elastase may deliver clinical benefit demonstrated by effects on the pathogenic processes that drive disease progression. These efficacy data, in combination with information about safety and tolerability, will be used to make progression decisions on further alvelestat (MPH966) development in AATD.

5.5. Justification for Dose

Single oral doses of alvelestat (MPH966) 2 mg to 150 mg have been evaluated in healthy subjects. Alvelestat (MPH966) is rapidly absorbed (median time to reach peak concentration 0.5 to 1.5 h), with a dose-proportional increase in systemic exposure. Multiple dosing shows no clinically relevant accumulation, and the PK profile was generally similar between healthy subjects and patients with lung disease. The PK data supported development of a model to simulate predicted doses and exposures for this study.

Alvelestat (MPH966) demonstrates dose-dependent inhibition of zymosan-stimulated neutrophil elastase activity in clinical studies[36]. Although at plasma alvelestat (MPH966) concentrations of >100 nM, neutrophil elastase inhibition was differentiated from that observed on placebo, plasma concentrations >300 nM, were required to give meaningful effects achieving a mean neutrophil elastase inhibition of 92%. Through observed and modelled PK, 120 mg bid is anticipated to provide a sufficient range of plasma exposures to achieve neutrophil elastase inhibition through PK levels >300 nM and will enable the concentration-effect relationship of alvelestat (MPH966) to be characterized.

As summarized below, the preclinical toxicology studies provide adequate safety margins for progressing to the 120 mg bid dose for 12 weeks in this study. The clinical safety experience with alvelestat to date has shown no signals that prevent progression of the proposed dose. The potential concern around transaminase elevation is considered monitorable and reversible. The safety of patients will be managed through the exclusion and inclusion criteria and safety monitoring approach taking into account the preclinical toxicology data and clinical safety information.

5.6. Preclinical Safety Studies

The safety margins for alvelestat (MPH966) have been evaluated in preclinical toxicology studies of up to 6 months in rats, 12 months in dogs, and 3 months in mice.

In rats, the no adverse effect level (NOAEL) was 370 mg/kg/day (1 month gavage) and 500 mg/kg/day (dietary 1 to 6 month studies). These were the highest doses studied, and no toxicity was observed.

In dog, 1-, 3-, and 12-month toxicology studies at doses of 1.2, 12, and 122 mg/kg showed limited effect at the intermediate and high doses, including small decreases in body weight, small increases in plasma creatinine and triglycerides, and minor effects on electrocardiogram (ECG) with a transient increase of the QTc by Van de Water's correction formula (QTcV) (<10%). The NOAEL was considered to be 122 mg/kg. In telemetered dogs at doses of 50 mg/kg (corresponding to a peak plasma concentration of 39.9 µM), a mean increase of 9% (individual increases of 8% to 14%) in QTcV was seen.

In mice, alvelestat (MPH966) was well tolerated at doses up to 2000 mg/kg. Histopathology indicated slight focal hepatic necrosis above control. Additional effects in liver included minor increase in glycogen vacuolation at 2000 mg/kg, slight hypertrophy in males at 1000 mg/kg, and increased incidence above control of inflammatory foci in females at 1000 mg/kg. A recovery group indicated possible reversibility, although the interpretation was difficult due to the low-grade change. Overall, focal necrosis is regarded as a non-specific response in mice, with no signs of progression or persistence and the relevance for humans is likely to be limited.

Preclinical toxicology findings, with the exception of QTc change, would be expected to be driven by area under the curve (AUC). Based on preclinical data, a human exposure limit corresponding to the AUC at the NOAEL (500 mg/kg) in the rat 6-month study (free AUC of 14.7 µM.h, corresponding to a total human AUC of 25.8 µM.h) is considered as acceptable in clinical studies. Significantly higher exposure levels have been evaluated in dogs for 12 months without any adverse effects.

The 120 mg bid dose in man is predicted to give an AUC of 11791 nM.h, approximately 53.8-fold lower than that at the NOAEL in the 12-month study in dogs and 21.5-fold lower than NOAEL in the 6 month study in rats. The AUC is predicted to be approximately 9-fold below the level where liver findings in mice (increase in background pathology of likely limited relevance to man) were seen.

An additional consideration when calculating the safety margins in man compared to toxicology studies is the differences in plasma protein binding seen across the species. Alvelestat (MPH966) plasma protein binding is less in humans than the other species investigated. For more detailed information on the toxicology and nonclinical safety studies conducted to date, please refer to the Investigator's Brochure.

5.7. Clinical Safety Studies

Clinical experience comes from 12 completed healthy volunteer and patient studies in which 1149 subjects have received at least 1 dose of alvelestat (MPH966). The highest single dose that has been given is 150 mg and the highest repeat dose is 240 mg bid (for 6 months). In clinical studies, 540 COPD patients have received 60 mg bid for 3 months and 22 patients with bronchiectasis and 26 patients with cystic fibrosis have received 60 mg bid for 28 days. Completed clinical studies to date have not revealed any adverse effects on blood pressure, ECG, hematology, or urinalysis that could be linked to any of the observations in toxicology studies. For clinical chemistry, there has been 1 patient with bronchiectasis with an instance of transient raised transaminases and 1 patient with cystic fibrosis with an increase in creatine kinase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase. In the studies in COPD patients, there were 14 instances where patients experienced raised ALT or AST to ≥3 × upper limit of normal (ULN) across the studied doses (alvelestat [MPH966] 5, 20, and 60 mg bid). Although definitive evidence of dose relationship was lacking, there was a suggestion of an increased incidence of liver transaminase elevations in patients on alvelestat (MPH966) for which there were no definitive alternative explanations. The highest transaminase elevations occurred in the

highest alvelestat (MPH966) dose (60 mg bid). There have been no cases of Hy's law related to alvelestat (MPH966) treatment reported in any of the studies to date (including up to 240 mg bid). The liver abnormalities noted to date with alvelestat, including from study MPH966-2-01 have been infrequent (only 5 liver-related adverse events have been reported and no SAE/AESI of liver abnormalities have been reported in study MPH966-2-01) and rarely above 3x ULN. Furthermore, all abnormal liver changes that have been observed have been reversible either with or without study drug interruption.

Alvelestat (MPH966) is currently being administered in 3 ongoing clinical studies. In a study in patients with bronchiolitis obliterans syndrome associated with chronic graft versus host disease following allogenic hematopoietic stem cell transplantation (NCT02669251), doses of up to 240 mg bid are being given. To date, 7 patients have received 240 mg bid dose for up to 6 months. One patient underwent dose reduction to 180 mg bid following a SAE of severe gastroenteritis and renal failure that was considered unrelated to alvelestat (MPH966). A second patient had elevation of transaminases with temporary treatment discontinuation on a dose of 60 mg bid. The event was considered related to alvelestat (MPH966), and drug was restarted at the dose of 60 mg bid at recovery following a 2-week temporary discontinuation. In an ongoing blinded study in AATD-related lung disease (MPH966-2-01, NCT03636347) 50 participants have been randomized to date to alvelestat (either 120 mg bid, 240 mg bid) or placebo (1:1:1) for 12 weeks duration, no safety signals have been identified.

For more detailed information on clinical safety studies conducted to date, please see additional details below and also refer to the current Investigator's Brochure.

5.8. Benefit-Risk Assessment

Patients with AATD-related lung disease present at a younger age and show more rapid progression that non-AATD COPD. The most common symptoms are shortness of breath, wheeze, chronic cough, and sputum production with a propensity for recurrent respiratory infections and bronchiectasis. Available therapies consist of chronic symptomatic treatment for COPD with bronchodilators and inhaled corticosteroids as well as management of acute exacerbations. None of these affect the progressive decline in lung function that may lead to severe structural damage, loss of lung function, and the need for lung volume reduction or transplantation in a select group. Augmentation with AAT protein is used in severe cases, with some evidence for effect on progression of lung damage. However, the costs of administration and the inconvenience for patients of a weekly intravenous therapy mean there is a high unmet need for more effective and convenient therapy.

Alvelestat (MPH966) is a potent, selective, and reversible inhibitor of human neutrophil elastase. Efficacy has been demonstrated in *in vivo* models of lung inflammation and elastase-induced lung injury[32]. Across clinical studies in COPD, cystic fibrosis, and bronchiectasis, there has been some evidence for effects on elastin breakdown, inflammatory biomarkers, and lung function. The extent of these effects was not consistent in all studies. This may be due to the heterogeneity of the pathogenesis and/or the doses used (maximum 60 mg bid) that would not be expected to adequately inhibit neutrophil elastase, allowing inflammatory "escape". Greater efficacy and clinical benefit is expected by targeting patients with AATD-related lung disease, in which neutrophil elastase is considered the key pathogenic driver, and by using a dose of alvelestat (MPH966) to maintain neutrophil elastase inhibition throughout the 24-hour period.

Alvelestat (MPH966) has been previously evaluated in clinical studies of up to 12 weeks in duration as a potential novel oral treatment to control the symptoms and exacerbations of COPD and reduce the progression and severity of the disease. Alvelestat (MPH966) has also been evaluated in studies in 2 other airway diseases characterized by neutrophilic inflammation: bronchiectasis[37] and cystic fibrosis[35]. Both studies were of 28 days in duration.

Headache was the most frequently reported AE in 8 of the 12 completed studies to date. In healthy volunteer studies, headache was reported more commonly in the avelestat (MPH966) group than the placebo group. Headache was generally mild in intensity (although 1 subject had severe headache), and there was no evidence of a dose–related relationship. In studies with cystic fibrosis and bronchiectasis, headache was also generally

reported more commonly in the alvelestat (MPH966) group compared to placebo. In the BREEZE (D0520C00012) and MISTRAL (D0520C00020) COPD studies, headache remained one of the most common AEs reported and was the most commonly reported treatment-related AEs (3 patients in the alvelestat [MPH966] 60 mg group) in the MISTRAL study. Headache has also been the most frequently reported adverse event particularly in the 240 mg bid dose group in study MHP966-2-01.

In the Phase 2b COPD program, there were 14 reported events of raised ALT or AST to ≥3 × ULN across the studied doses (alvelestat [MPH966]) 5, 20, and 60 mg bid); there were no cases of Hy's law during the treatment period. Although definitive evidence of dose relationship was lacking, there was a suggestion of an increased incidence of liver transaminase elevations in patients on alvelestat (MPH966) for which there were no definitive alternative explanations. The highest transaminase elevations occurred in the highest alvelestat (MPH966) dose (60 mg bid). Elevations in hepatic biochemistry assessments (transaminases) observed in patients in the alvelestat (MPH966) studies were confounded by alcohol or by concomitant medication, but an effect of alvelestat (MPH966) cannot be excluded at this time. A significant proportion (49%) of adult patients with Pi*ZZ AATD have been shown to have small increases in levels of ALT[41] and some develop cirrhosis. It is therefore possible that patients with AATD may be more susceptible to liver function AEs. Therefore, exclusion criteria for clinically relevant hepatic fibrosis, in-study monitoring, and study treatment discontinuation criteria are in place to protect the safety of the subjects and liver abnormalities are included as adverse events of special interest (AESI).

There were no other clinically relevant changes in clinical chemistry data in the completed studies with alvelestat (MPH966). There were also no clinically relevant changes in urinalysis were observed in the completed studies with alvelestat (MPH966). There were no changes in ECG or vital signs data that indicated a clear treatment effect of alvelestat (MPH966). However, in light of the minor changes in QTc in the dog toxicology, exclusion criteria and regular ECG monitoring at the time of peak concentrations of study treatment and discontinuation criteria will be applied in the study. Clinically significant changes in ECG are also included as an AESI.

Mutations in *ELAINE*, the gene that encodes neutrophil elastase, are associated with cyclical neutropenia, severe congenital neutropenia, and susceptibility to recurrent infection. To date, no increases in rate or severity of infection or neutropenia have been reported with alvelestat. However, for this initial study in AATD, patients with neutropenia will be excluded, new onset of neutropenia will constitute a patient stopping criterion, and infections will be an AESI to monitor the safety profile as higher doses are explored in a new population.

There have been no liver abnormalities in the current study, and those noted to date with alvelestat in study MPH966-2-01, also in alpha-1 antitrypsin deficiency, have also been infrequent (only 5 liver-related adverse events have been reported and no SAE/AESI) and rarely above 3x ULN. Furthermore, all abnormal liver changes that have been observed have been reversible either with or without study drug interruption up to doses of 240 mg bid for 12 weeks.

At present, based on the cumulative review of the data and with no new safety issues seen in the ongoing studies with dosing up to 240 mg bid of alvelestat (MPH966), the safety profile of alvelestat (MPH966) remains unchanged and the benefit-risk balance continues to support the clinical development of alvelestat (MPH966). The potential or theoretical safety concerns on liver function, QTc changes, and susceptibility to infection are manageable through exclusion criteria, monitoring, study treatment discontinuation criteria, and independent safety review through the DSMB, as detailed in Appendix 7.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of alvelestat (MPH966) may be found in the current version of the alvelestat (MPH966) Investigator's Brochure.

6. Study Population

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, are not permitted.

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if ALL of the following criteria apply:

Type of Participant and Disease Characteristics

1. Capable of giving signed informed consent as described in Appendix 3, which includes compliance with the requirements and restrictions listed in the informed consent form and in this protocol
2. Age ≥18 and ≤80 years
3. Patients with a confirmed diagnosis of AATD: Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low (serum AAT level <11 µM or <57.2 mg/dL) or functionally impaired AAT including "F" or "I" mutations.
4. FEV_1 ≥25% predicted
5. Patients will be eligible if they are either a) are not currently receiving augmentation treatment and have not received augmentation in the 12 weeks prior to screening or b) have received weekly infusions of augmentation at 60 mg/kg for at least 12 weeks prior to screening and intend to continue augmentation through the study period.
6. Male or female sex
    a. Male participants must agree to use a highly effective contraception as detailed in Appendix 5 during the treatment period and for at least 4 days after the last dose of study treatment and refrain from donating sperm during this period
    b. Female participants are eligible to participate if not pregnant; not breastfeeding; and at least one of the following conditions is met:
        i. Not a woman of childbearing potential as defined in Appendix 5
        OR
        ii. A woman of childbearing potential who agrees to follow the contraceptive guidance in Appendix 5. During the treatment phase and for at least 4 days after the last dose of study medication.

6.2. Exclusion Criteria

Participants are excluded from the study if ANY of the following criteria apply:

Excluded Medical Conditions

1. Subjects with Pi*MZ, Pi*FM, Pi*MS, Pi*SS, or other AATD phenotypes/genotypes not known to be independently associated with emphysema.
2. Any clinically diagnosed lung disease other than COPD such as diffuse interstitial lung diseases, cystic fibrosis, or clinically significant bronchiectasis as determined by the Investigator
3. Acute exacerbation of underlying lung disease requiring oral steroids and/or antibiotics within 4 weeks of baseline
4. Acute or chronic hepatitis, including hepatitis B, hepatitis C (positive serologies, including hepatitis B and C antibody)
5. HIV infection or other immunodeficiency or with an absolute neutrophil count ≤1.0×10^9/L
6. Abnormal liver biochemistry (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) >1.5 × upper limit of normal or total bilirubin > upper limit of normal (unless Gilbert's disease with normal conjugated bilirubin)

7. Any of the following laboratory abnormalities are present at baseline:
    a. Platelet count <150×10^9/L
    b. Serum albumin ≤ 3.5 g/dL
    c. INR ≥1.2
    d. CPK ≥ ULN.
8. History or current evidence of cirrhosis (on biopsy or imaging), esophageal varices, ascites or hepatic encephalopathy.
9. Evidence of other forms of chronic liver disease based on diagnostic testing as per the guidelines (i.e. autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, Hemochromatosis or iron overload).
10. Patients with nonalcoholic fatty liver disease (NAFLD) as diagnosed by any imaging modality (or use of drugs associated with NAFLD for more than 2 weeks in the year prior to screening).
11. Subjects with a history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening, defined as average of >20g/ day in female subjects and >30g/ day in male subjects.
12. Fibrosis-4 (FIB-4) score >3.25
13. Any of the following cardiovascular conditions within 6 months prior to the screening visit:
    a. Myocardial infarction or unstable angina
    b. Coronary artery bypass surgery, balloon angioplasty, percutaneous coronary intervention, or carotid revascularization procedure
    c. Uncontrolled hypertension
    d. Stroke or transient ischemic attack
14. Congestive heart failure (New York Heart Association III/IV) with left ventricular ejection fraction < 40%
15. Any clinically significant 12-lead electrocardiogram abnormalities at screening or baseline, including corrected QT interval by Fridericia's correction method >450 ms or history of significant cardiac dysrhythmia, including long QT syndrome
16. History of cancer within the last 5 years, except for well-treated basal cell carcinoma and squamous cell carcinoma of the skin
17. Other documented comorbidities or laboratory abnormalities that in the opinion of the Investigator could affect the outcome of the study assessments, participant safety, or ability of the participant to comply with the requirements of the protocol

Excluded Prior/Concomitant Therapy

18. Daily use of prednisone (>10mg daily), or other systemic glucocorticoids at comparable or higher equivalent dose, or use of other immunosuppressant therapies are prohibited
19. Immunomodulating monoclonal antibodies within 6 months prior to screening are prohibited
20. Daily use of non-steroidal anti-inflammatory drugs (NSAIDs) is prohibited. Daily use of acetaminophen up to 2 g per day and aspirin up to 325 mg per day is permitted.
21. Initiation of drugs known for hepatotoxic potential within the 28 days prior to screening including but not limited to: statins, NSAIDS, amoxicillin/clavulanate, PDE inhibitors (theophylline, roflumilast), and anti-epileptics. Subjects on established treatment for more than 28 days prior to screening will not be excluded. Requirement for medications mainly metabolized by CYP2C9 and with narrow therapeutic index (eg, warfarin, phenytoin) is prohibited

Excluded Prior/Concurrent Clinical Study Experience

22. Participation in any clinical investigation using medical devices or non-biologic treatments within 4 weeks or 5 half-lives of the drug (whichever is longer) prior to the initial dosing (or longer if required by local regulations) is prohibited
23. Participation in any clinical investigation using biologic treatment within 6 months of screening is prohibited
24. Previous participation in a gene therapy study for AATD at any time is prohibited

Other Exclusions

25. History of hypersensitivity to alvelestat (MPH966) or any of its excipients or the class of neutrophil elastase inhibitors
26. Known hypersensitivity to medications used in the study procedures (e.g. midazolam, fentanyl, and lidocaine for bronchoscopy)

6.3. Lifestyle Restrictions

6.3.1. Meals and Dietary Restrictions

There are no specific restrictions in respect of mealtimes and administration of study treatment.

6.3.2. Alcohol and Tobacco

Tobacco cessation is encouraged but is not required for the study duration.

Participants should avoid alcohol during the 12 week active study period.

6.3.3. Activity

Subjects should not participate in unaccustomed or more vigorous exercise than usual routine in the 48 hours before each blood sample for laboratory safety tests.

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, available eligibility criteria, and any SAEs. Individuals who do not meet the criteria for participation in this study (screen failures) may be rescreened after discussion with and approval by the Data Coordinating Center (DCC) and/or Principal Investigator (PI).

The **screening period may be extended beyond 28 days** in the following circumstances after discussion with and approval of the DCC and/or PI:

- All screening inclusion/exclusion criteria are met but the participant's schedule, including genetic testing, prevented randomization within the screening period.
- The participant experiences an acute exacerbation of COPD during the screening period.

Additionally, individual laboratory parameters or screening tests may be repeated, within the screening period, if the result is considered aberrant or not clinically consistent with the patient's health status. Decisions to re-test should be made in conjunction with the study PI.

Participants who have individual laboratory parameters re-tested should have the results entered in the electronic case report form (eCRF).

7. Treatments

Study treatment is defined as the investigational treatment or placebo.

7.1. Treatments Administered

Study Treatment Name:	Alvelestat 120 mg	Placebo
Dosage Formulation:	Tablet	Tablet
Unit Dose Strength(s) / Dosage Level(s):	4 × 30 mg Alvelestat	4 × 30 mg Placebo
Route of Administration	Oral	Oral
Dosing Instructions:	To be taken bid, 12 hours apart and with water	
Packaging and Labeling:	Study treatment will be provided in boxes. Each box will contain treatment for 7 days and will be labeled as required per U.S. regulations.	
Manufacturer:	Mereo BioPharma 4 Ltd., 1 Cavendish Place, London, W1G 0QF, UK	

7.2. Method of Treatment Assignment

All participants will be centrally randomized 1:1 to the 120 mg and placebo arms using an interactive web response system (IWRS). A unique number will be assigned to each participant and will be linked to randomization numbers using a randomization list produced by the DCC. These randomization numbers will be linked to the 2 treatment regimens, and the randomization scheme will be stratified according to genotype (Pi*ZZ, Pi*SZ, Pi*(Null) (Null)) and clinical center.

7.3. Blinding

This is double-blind study. The investigators, participants, Mereo and NCATS will remain blinded to the study treatment allocation until the end of the study. The randomization list will be kept secure from the study team, investigators, and participants throughout the conduct of the study and until unblinding is authorized by the DCC. The DSMB will be unblinded according to the DSMB charter.

The IWRS will be programmed with blind-breaking instructions. The study blind may be broken for a medical emergency in which the knowledge of the specific blinded study treatment will affect the immediate management of the participant's condition. In this case, the DCC, the NCATS Medical Monitor, and Mereo must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form (CRF), as applicable.

7.4. Preparation/Handling/Storage/Accountability

Only participants enrolled in the study may receive study treatment, and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatment are provided in the Manual of Procedures (MOP).

7.5. Treatment Compliance

The per-protocol dosage, timing, and mode of administration of study treatment may not be changed. Any departures from the intended regimen must be recorded in the electronic eCRF and reported to the DCC.

7.6. Concomitant Therapy

Treatment for Respiratory Disease

Bronchodilators are allowed as prescribed. However, they should be withheld for the required time before spirometry as detailed in the MOP.

Other regular medications for treatment of airways disease (e.g., inhaled bronchodilators or inhaled corticosteroids) are permitted provided the subject has been on a stable dose for 1 month before baseline.

Systemic Corticosteroids

Regular systemic steroids are not permitted during the study, unless clinically indicated to treat acute exacerbation.

Vaccines

Patients should be vaccinated against pneumococcus and receive annual influenza vaccination.

CYP2C9 Substrates

MPH966 has a weak potential to inhibit CYP2C9. Therefore, medications that are metabolized mainly by CYP2C9 and have a narrow therapeutic index are prohibited. Narrow therapeutic index CYP2C9 substrates include warfarin and phenytoin (a complete list is included in the MOP or online at http://medicine.iupui.edu/clinpharm/ddis/main-table/).

Enhanced clinical monitoring in patients receiving sensitive/moderate sensitive CYP2C9 for enhanced clinical effect should be considered. Moderate sensitive substrates include tolbutamide and glimepiride.

Therapies Known to Cause Liver Function Abnormalities

Daily treatment with acetaminophen at doses greater than 2 g per day or non-steroidal anti-inflammatory drugs (NSAIDs) is not permitted during the study. Acetaminophen at doses of up to 2 g per day and aspirin up to 325 mg per day are permitted during the study.

Initiation of drugs known for hepatotoxic potential within the 28 days prior to screening including but not limited to: statins, amoxicillin/clavulanate, PDE inhibitors (theophylline, roflumilast), and anti-epileptics is exclusionary. Subjects on established treatment for more than 28 days prior to screening will not be excluded.

During the course of the study, if medications are required, a concomitant medication's hepatotoxicity and alternative treatments, if possible, should be considered. In the event that no alternatives are available and the medication is required to treat the medical condition, the Investigator should discuss the subjects' ongoing participation in the trial with the study PI and/or DCC at the earliest possible opportunity.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The DCC and/or study PI should be contacted if there are any questions regarding concomitant or prior therapy.

7.7. Treatment After the End of the Study

Alvelestat (MPH966) will not be provided after the end of the study.

8. Discontinuation Criteria

8.1. Discontinuation of Study Treatment

In considering discontinuation of the study treatment, the Investigator should give particular consideration of the following:

Liver Test Abnormalities

The following monitoring schedule is proposed when a case of acute liver function abnormalities or worsening of a liver function abnormality is reported:

- If ALT or AST are between 1.5 to 3 × ULN, regular monitoring (every 2 weeks at a minimum) is required until event resolution and patient can **continue on study treatment** during this period.

- If ALT or AST are ≥3 × ULN, repeat the test within 48 to 72 hours and the **study treatment will be temporarily discontinued** while awaiting repeat tests results; if the repeat tests is again ≥3 × ULN or ≥2 × pre-treatment level, testing needs to be repeated at least twice weekly until event resolution or a return to baseline value while the study treatment is **withheld**.

- If AST or ALT are ≥5x ULN, a repeat value should be obtained within 24-48 hours. If the repeat ALT and AST values is ≥5 x ULN, the drug should be discontinued and the patient followed until the laboratory values normalize or stabilize, in addition to initiating potential (Drug Induced Liver Injury) DILI evaluation.

Discontinue Alvelestat (MPH966) for the following hepatic adverse events:

- Regardless of the magnitude of the transaminase elevation, if the bilirubin is elevated (any level) in addition to the presence of signs and symptom(s) such as rash, eosinophilia, nausea, vomiting, or right upper quadrant pain,

- INR ≥1.5 and TB ≥2x ULN, irrespective of the magnitude of ALT or AST elevation.

- TB elevation ≥2x ULN, and ALP ≥2x ULN for cholestatic liver injury.

For each scenario, initiate potential DILI evaluation for alternative etiologies and obtain a hepatology consult. Liver biopsy should be considered for DILI. The complete liver profile including PT/INR must be repeated within 48-72 hours after first abnormal value is obtained. Follow the patient until laboratory parameters stabilize or normalize. Study medication can be restarted only if an alternative etiology is "definitively" identified and liver tests have returned to baseline. Testing for alternative etiologies can include serology tests (hepatitis A, hepatitis B, hepatitis C, Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Herpes Simplex Virus (HSV), varicella zoster virus (VZV), parvovirus, toxoplasmosis), imaging, and pathology assessments (creatine phosphokinase, clotting) as appropriate to the clinical situation.

Clinically significant liver function abnormalities should be reported as an AE/AESI.

When the following abnormal liver test conditions are met, study treatment **MUST** be discontinued, the DCC and study PI informed, and further steps discussed:

- ALT/AST >5 × ULN on 2 consecutive tests within 24 to 48 hours **OR**
- ALT or AST >3 × ULN and total bilirubin >2 × ULN **OR**
- ALT or AST >3 × ULN and international normalized ratio (INR) >1.5 **OR**
- ALT or AST >3 × ULN the appearance of worsening fatigue, nausea, vomiting, right upper quadrant pain/tenderness, fever, rash, or eosinophilia

ECG Abnormalities

If a clinically significant finding is identified (including but not limited to changes from baseline in QTcF after enrolment), the Investigator or qualified designee will determine if the participant can continue in the study and if any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

When the following ECG change conditions are met, study treatment **MUST** be discontinued, the DCC and study PI informed, and further steps discussed:

- Absolute QTcF >500 ms or >60 ms increase from baseline as confirmed with 3 consecutive ECGs taken in a 30-minute period with at least 5 minutes between each ECG. The mean of the 3 ECGs will be used for decision making.

Other Laboratory Parameters

Study treatment **MUST** be discontinued if the following laboratory parameters criteria are met:

- Absolute neutrophil count less than or equal to 1.0 x 10^9/L on 2 consecutive repeat tests within 48 to 72 hours.

Other

Study treatment **MUST** be discontinued if the following other criteria are met:

- Significant safety issues (as judged by the Site Investigator). This includes AEs considered unacceptable by the patient and/or the Investigator
- Any other protocol deviation that results in a significant risk to subject's safety
- Pregnancy as confirmed by positive urine test at any time during the study
- Withdrawal of informed consent

Participants may voluntarily discontinue investigational treatment for any reason at any time. Participant decision on discontinuation of study treatment does not imply an automatic withdrawal from the study.

Refer to the Schedule of Activities (SoA) for data to be collected at the time of treatment discontinuation and follow-up and for any further evaluations that need to be completed.

8.1.1. Temporary Discontinuation

Study treatment dosing may be temporarily suspended in the event of:

- Clinically important laboratory abnormalities
- Other intercurrent illnesses including acute exacerbations of COPD, major surgery, or gastrointestinal problems
- Use of prohibited treatment
- Any other protocol deviation that results in a significant risk to the participant's safety

After a laboratory abnormality leading to a delay of dosing normalizes sufficiently, study treatment may resume at the discretion of the Site Investigator in consultation with the Study PI as needed. Similarly, study treatment may resume after the medication leading to suspension of dosing is discontinued. A decision to discontinue study treatment and/or to reinstitute study treatment should be conveyed to the Study PI and the DCC. The Investigator may suspend study treatment at any time, without consultation with the Study PI if the urgency of the situation requires immediate action and if this is determined to be in the participant's best interest. However, the Study PI and DCC should be contacted as soon as possible in any case of study treatment discontinuation.

8.1.2. Re-challenge

Resumption of study treatment after temporary discontinuation should always be discussed with the DCC and study PI.

Re-challenge should be performed one time only if the study medication was held due to AST or ALT ≥3x ULN. Re-challenge should only occur after the ALT or AST return to pretreatment baseline value.

In the specific case of liver tests meeting the study treatment discontinuation criteria in Section 8.1, no re-challenge is permitted.

8.2. Discontinuation From the Study

The Investigator should consider the following when withdrawing a participant from the study:

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the DCC may retain and continue to use any data collected before such the withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records and notify the DCC.

8.3. Lost to Follow-up

A participant will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- In cases in which the participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and mail a certified letter to the participant's last known mailing address. These contact attempts should be documented in the participant's file.

- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. Study Assessments and Procedures

- Study procedures and their timing are summarized in the SOA.

- Adherence to the study design requirements, including those specified in the SOA, is essential and required for study conduct.

- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

- .

- Sample handling: Routine bloodwork and laboratory measurements used for safety monitoring and eligibility assessment (CMP, coagulation measurements, CBC, pregnancy testing, etc.) will be processed and measured using local laboratory. Samples (blood, sputum, BAL) used for PK or biomarker assessment will undergo initial processing at the local site but will be shipped to a central lab (Dr. Wells) at UAB for curation and analysis. The procedure is detailed in the MOP.

- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

- Biomarker samples (blood, sputum, and BAL) will be stored for up to 60 months after the end of the study.

9.1. Key Screening Assessments

FIB-4 Score

The FIB-4 test combines age with 3 standard biochemical values (platelets, ALT, and AST) as a non-invasive test to assess fibrosis and enable exclusion of participants who may be at greater risk of liver AEs (https://www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis). The following formula will be used:

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST Level (U/L)}}{\text{Platelet Count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$$

Using a cut-off value of 1.45, a FIB-4 score <1.45 has been shown to have a negative predictive value of 90% for advanced fibrosis. In contrast, a FIB-4 >3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis[42]. FIB-4 index has been shown to be superior to other scoring systems for differentiating between advanced and mild fibrosis.

9.2. Efficacy Assessments

9.2.1. Blood, Sputum, and Bronchoalveolar Lavage Biomarkers

Blood

Blood will be collected as per the SOA, and samples will be collected and processed as described by the MOP.

Sputum

Sputum will be collected from induced samples (refer to the MOP for processing details). Sputum induction will not be undertaken if the baseline FEV_1 for the procedure is less than 1 L. Further details of sputum induction will be included in the MOP. Sputum induction is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

Bronchoalveolar Lavage (BAL)

BAL will be collected in the subset of participants who agree to participate in the bronchoscopy sub-study. Samples will be collected at two time points as per the SOA, and samples will be collected and processed as described by the MOP. Bronchoscopy and BAL is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

Blood, sputum, and BAL efficacy biomarkers (see Table below) will be taken at the time points as detailed in the SoA to evaluate their association with the observed mechanism of action responses to neutrophil elastase inhibition, including response of neutrophil elastase activity, inflammation, and lung damage to alvelestat (MPH966).

The samples will be collected and processed as described in the MOP.

Samples may be stored for a maximum of 60 months following the end of study at a facility selected by the Sponsor to enable further analysis of biomarker responses to alvelestat (MPH966).

Biomarkers of Efficacy Collected in Blood and Sputum

Biomarker	Blood	Sputum	BAL
Neutrophil Elastase Related Biomarkers			
Desmosine/Isodesmosine	X	X	X
Aα-Val^{360}	X	X	X
NE	X	X	X
EL-NE	X	X	X
Proteinase 3	X		X
Cathepsin B	X		X
Lung Damage Biomarkers			
EL-CG	X		X
EL-P3	X		X
PGP	X	X	X
C6M	X		X
C1M	X		X
PRO-C6	X		X
Inflammatory Biomarkers			
Hs-CRP	X		
IL-6	X	X	X
IL-8	X	X	X
RANTES	X	X	
IL-1β	X	X	
Fibrinogen	X		
LTB-4		X	X
MMPs	X	X	X
MPO	X	X	X

In addition, samples will be stored and analysis may be performed on additional biomarker variants thought to play a role in neutrophil elastase–induced lung damage to evaluate their association with observed neutrophil activation responses to alvelestat (MPH966).

9.2.2. SGRQ-C

The St. George's Respiratory Questionnaire (SGRQ-C) will be administered at time points as detailed in the SoA. This is a questionnaire that measures health impairment in patients with COPD[43]. It consists of 3 parts: a symptom score, an activity score, and an impact score. A total score is also produced.

9.2.3. COPD Assessment Test (CAT)

The CAT is an 8 item questionnaire used to report health status in COPD[44]. It will be administered at time points as shown in the SOA.

9.2.4. Modified Medical Research Council (MMRC)

The MMRC will be administered at time points as detailed in the SoA and is used to measure dyspnea on a 5-point scale[45].

9.2.5. San Diego Breath Questionnaire (SOBQ)

The SOBQ assesses patient reported dyspnea[46]. The SOBQ will be administered at time points as detailed in the SoA.

9.2.6. Pulmonary Function Testing/Spirometry

Pulmonary function testing (FEV_1, FVC, FEV_1/FVC, and maximal mid-expiratory flow) will be performed at the times specified in the SoA according to ATS guidelines. Further details on the spirometry procedure can be found in the Specific MOP. Spirometry is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

9.2.7. Daily respiratory symptom scores and peak flow by electronic diary

Participants will complete a 2 week baseline period of diary entries after screening and prior to randomization to establish a baseline. Participants will then complete the daily diary throughout the 16 week treatment period. Study sites will review diary entries at each clinic visit to confirm compliance and query possible adverse events.

9.3. Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

9.3.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, integumentary, and neurological systems. Height and weight will also be measured and recorded.

- A brief physical examination will include, at a minimum, assessments of the lungs, heart, abdomen, and skin.

- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.3.2. Vital Signs

Vital signs will be measured in a semi-supine position after 5 minutes of rest and will include temperature, systolic and diastolic blood pressure, heart rate, respiratory rate, and pulse oximetry.

- Oral or tympanic temperature.

- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).

- Pulse oximetry will be measured using a sensor placed on the participant's fingertip or earlobe. Oxygen saturation (SpO2) will be recorded.

Electrocardiograms

- 12-lead ECG will be obtained as outlined in the SoA using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 8.1 for QTc withdrawal criteria and additional QTc readings that may be necessary.

- Single ECGs will be performed at all scheduled time points, with the exception of when a change from baseline occurs that meets drug discontinuation criteria (Section 8.1). In this situation, a triplicate measurement is required to confirm the finding and for discontinuation decisions.

- When a triplicate ECG is required, 3 consecutive ECG tracings should be taken in a 30-minute period with at least 5 minutes between each ECG.

- QTc value will be calculated using the Fridericia formula ($QTc=QT/3\sqrt{RR}$).

9.3.3. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SOA for the timing and frequency.

- The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.

- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 28 days after the last dose of study treatment should be repeated until the values return to normal or baseline, with a frequency based on the judgement of the Site Investigator. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the DCC notified.

  The monitoring schedule proposed for a case of acute liver function abnormalities or worsening of a liver function abnormality is reported is listed in Section 8.1 (Liver Test Abnormalities).

- Clinically significant liver function abnormalities should be reported as an AE/AESI.

- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the MOP and the SOA.

9.3.4. Data Safety Monitoring Board

Safety will be regularly reviewed by an independent DSMB who will have the option to unblind masked group data as necessary.

At any time, the DSMB may choose to recommend pausing, modifying, or stopping enrolment.

Full details of composition, operational aspects, and data to be reviewed and recommendations to be made by the DSMB will be described in the DSMB charter.

9.4. Adverse Events

The definitions of an AE, SAE, SUSAR, and UP can be found in Appendix 4.
An AE will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study treatment (see Section 8.1).

9.4.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the signing of the ICF until the follow-up visit at the time points specified in the SoA.

All SAEs will be recorded and reported to the DCC within 24 hours, as indicated in Appendix 4.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.4.2. Follow-up of an AE and/or SAE

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and non-serious AESIs (as defined in Section 9.4.3), will be followed until resolution, until stabilization, until the event is otherwise explained, or until the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Appendix 4.

9.4.3. Adverse Events of Special Interest

Some AEs, regardless of their severity or outcome, will be expedited due to the relevance for subject safety or study treatment safety profile. These events should be reported to the DCC within 24 hours, as indicated in Appendix 4. The AESIs for alvelestat (MPH966) are:

- Liver function abnormalities defined as:
  a) Any occurrence of (i) ALT or AST elevations >5 × ULN or (ii) ALT or AST >3 × ULN and total bilirubin >2 × ULN or INR >1.5 or the appearance of worsening fatigue, nausea, vomiting, RUQ pain/tenderness, fever, rash, or eosinophilia.

  b) Any occurrence of <5 × ULN or ALT or AST <3 × ULN and total bilirubin <2 × ULN where the tests have been repeated within 48 to 72 hours and the elevation confirmed on at least 2 separate occasions.

For participant safety and to ensure that the hepatotoxic potential of the study treatment to be determined, a standardized procedure for identification, monitoring, and evaluation of liver events must be followed as outlined in Section 9.3.3 and Section 8.1.

- ECG/Cardiac Events

If a study subject experiences any of the following cardiovascular events, the event should be reported immediately as an AESI and the study treatment discontinued:

a) Absolute QTcF >500 ms or >60 ms increase from baseline.

b) Any clinically significant cardiac abnormality on ECG.

- Infection

Any new infection that requires the use of systemic antimicrobial treatment (antibiotics, anti-viral, anti-fungal, or anti-mycobacterial) should be investigated with clinically relevant diagnostic tests.

Acute exacerbations of COPD are considered AESI.

- Neutropenia

a) Absolute neutrophil count less than or equal to 1.0×10^9/L on 2 consecutive repeat tests within 48 to 72 hours.

9.4.4. Regulatory Reporting Requirements for SAE

- Prompt notification by the Investigator to the DCC of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

- The DCC, the NCATS Medical Monitor, as well as Mereo have a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and will be forwarded to Investigators as necessary.

- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g. summary or listing of SAEs) will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.4.5. Pregnancy

- Details of all pregnancies in female participants and female partners of male participants will be collected after the start of study treatment and until 5 half-lives (4 days) after the last dose.

- If a pregnancy is reported, the Investigator should inform the DCC within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 5.

- Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

Partner Pregnancies

Pregnancy outcomes must be collected for the female partners of the subjects who took study treatment in this study. Pregnancy itself is not regarded as an AE unless there is suspicion that the study treatment may have interfered with the effectiveness of a contraceptive medication. If a pregnancy is reported for a subject's partner, study treatment will be immediately discontinued. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed and documented. A follow-up report should be sent with any new information regarding the pregnancy and the outcome of the birth.

9.5. Treatment of Overdose

For this study, any dose of alvelestat (MPH966) greater than 120 mg bid within a 24-hour time period will be considered an overdose.

There is no specific treatment for an overdose. Any treatment should be supportive as indicated by the subject's condition.

In the event of an overdose, the Investigator/treating physician should:

1. Contact the DCC and study PI immediately.

2. Closely monitor the participant for AE/SAE and laboratory abnormalities until alvelestat (MPH966) can no longer be detected systemically (at least 3 days).

3. Obtain a plasma sample for PK analysis within 3 days from the date of the last dose of study treatment if requested by the DCC and/or study PI (determined on a case-by-case basis).

4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Study PI as necessary based on the clinical evaluation of the participant.

9.6. Pharmacokinetics

Sparse PK sampling will be performed before and after study treatment dosing as detailed in the SoA and MOP.

- Blood samples of approximately 3 mL will be collected for measurement of blood concentrations of alvelestat (MPH966) as specified in the SoA. Instructions for the collection and handling of biological samples will be provided by the DCC. The actual date and time (24-hour clock time) of each sample will be recorded.

9.7. Pharmacodynamics

Pharmacocodynamic markers form the primary, and some secondary and exploratory efficacy endpoints in this study and are covered in Section 9.2.1.

10. Statistical Considerations

10.1. Sample Size Determination

Over the course of the study, approximately 60 participants will be randomized in a 1:1 ratio to receive one of the following: 120mg alvelestat (MPH966) BID or placebo. Randomization will be stratified by study site and by AAT genotype/phenotype.

The study will be powered for within-individual change in plasma desmosine/isodesmosine as the primary endpoint at Week 12 compared to baseline. A sample size of 30 participants in the MPH966 treatment group will give us >90% power to detect a 15% mean decrease of plasma DES from baseline to 12 weeks follow-up in patients treated with MPH966 (within subject change) using a two-sided paired t-test at α=0.05. This 15% mean decrease has been observed in trials of standard dose augmentation versus placebo [31]. A recent study of double dose versus single dose augmentation revealed a within subject decrease in plasma DES from 0.42 +/-0.03 ng/ml to 0.38 +/- 0.03 ng/ml [12]. We will have 80% power to detect a comparable difference in DES in those who have alvelestat added to augmentation.

For comparing the mean change in DES between the two groups (placebo vs. MPH966), and assuming that there is no placebo effect over 12 weeks, a two-sided two sample t-test at α=0.05 with a sample size of 60 (30 per group) reveals that we will have 82% power to detect a 15% difference in mean change in plasma DES.

Other secondary endpoints (BAL and sputum DES, other serum, sputum and BAL inflammatory markers, patient-reported outcomes, and pulmonary function) will be examined within and between group with paired t-tests or mixed effect modeling as appropriate.

We will also examine the association between DES at baseline and response to treatment as measured by change in pulmonary function (FEV1) over 12 weeks within the MPH966 treatment group. With a sample size of 30, we will have 80% power to detect a 0.12 regression correlation coefficient of DES at baseline in predicting the change of FEV1 over 12 weeks when the standard deviation of DES is 0.10 and α=0.05.

By including a 10% attrition rate over 12 weeks, the required sample size is inflated to 66 (33 per group) to achieve the estimated powers. If we observe attrition rate less than we expected, we will have more statistical power to detect the expected differences. As one interim analysis will be conducted only for safety concerns (see Data Safety and Monitoring Plan), no adjustments to the Type I error is considered for estimating statistical powers.

10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled Set	The Enrolled Set will include all participants who sign the ICF.
Randomized Set	The Randomized Set will include all subjects who signed the ICF and were subsequently randomized into the study, regardless of study treatment administration.
Full Analysis Set	The Full Analysis Set will serve as the primary population for the analysis of efficacy and will consist of all randomized subjects who took at least 1 dose of double-blind study treatment and have at least 1 evaluable change from baseline in plasma desmosine/isodesmosine levels. Subjects will be analyzed according to randomized treatment.

Population	Description
Per-Protocol Set	The Per-Protocol Set includes all participants from the Full Analysis Set who have been treated according to the protocol and fulfil the following criteria: 1. All inclusion/exclusion criteria satisfied 2. Absence of relevant protocol violations with respect to factors likely to affect the efficacy of treatment where the nature of protocol violation will be defined before breaking the blind 3. Adequate study medication compliance, which will be determined before breaking the blind Subjects will be analysed according to randomized treatment.
Safety Set	All randomized participants who take at least 1 dose of study treatment. Participants will be analysed according to randomized treatment.
PK Set	The PK Set will include all participants in the Safety Set who have at least 1 evaluable serum concentration.

10.3. Statistical Analyses

The statistical analysis will be performed using SAS® version 9.4 or higher. The main population for efficacy analysis will be the Full Analysis Set; supportive analyses will also be performed using the Per-Protocol Set.

Continuous data will be presented using descriptive summaries (e.g., mean, standard deviation, minimum, maximum, median, lower quartile, and upper quartile). Categorical variables will be presented by the number of observations and relative (%) frequency.
Unless otherwise stated, baseline value for any variable will be the last value taken prior to the first dose of study medication.

Unless otherwise stated, all statistical tests will be 2-sided and conducted at the 5% level. All presented confidence intervals will be 2-sided 95% confidence intervals.

The Statistical Analysis Plan (SAP) will be developed and finalized before database lock and will describe the selection of participants to be included in the analyses and procedures for accounting for missing, unused, and spurious data. Below is a summary of planned statistical analyses of the primary and secondary endpoints.

10.3.1. Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	Within individual % change from baseline in plasma desmosine/isodesmosine levels at 12 weeks will be calculated. An analysis of covariance (ANCOVA) model with baseline plasma desmosine/isodesmosine levels as a covariate and treatment and sputum strata as factors will be used to generate least squared means and least squared mean differences between treatment groups.
Secondary	The biomarker correlation analysis will be done using stepwise approach: The parameters in BAL, sputum, and blood during treatment will be compared to the baseline values for active and placebo patients separately to evaluate natural variability of the markers. The time-matched biomarker values will be compared between placebo and active groups to assess the differences outside natural variability and time to significant change for the responsive biomarkers.

Endpoint	Statistical Analysis Methods
	Multiple models, including non-parametric models, will be considered within this frame incorporating different covariates and data transformations. PD parameters such as area under the effect-time curve will also be considered. Principal component multifactorial analysis and/or cluster analysis may be employed to simplify the comparison and to explore the treatment effect. Decisions will be made on review of data and prior to database lock. Correlation of select biomarkers shown to be responsive to treatment with time-matched drug concentrations and PK parameters, if available, may be used to evaluate exposure-response relationship.
Exploratory	Observed values and change from baseline in FEV_1, FVC, FEV_1/FVC (total and percent predicted) and maximal mid-expiratory flow will be presented by treatment group and study visit. Change from baseline at Week 12 will be analysed using an ANCOVA model. Observed values and change from baseline for the total and component scores of the SGRQ-C will be presented by treatment group and study visit. Change from baseline in total score at Week 12 will be analysed using an ANCOVA model. Correlation of select biomarkers shown to be responsive to treatment with time-matched drug concentrations and PK parameters, if available, will be used to evaluate exposure-response relationship.

10.3.2. Safety Analyses

All safety analyses will be performed on the Safety Set.

Endpoint	Statistical Analysis Methods
Primary	AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Summaries will be by system organ class and preferred term. Treatment-emergent AEs are defined as any AE occurring or worsening on or after the first dose of study medication. If a participant experiences the same preferred term multiple times, the event will be counted only once overall and by the greatest severity. The frequency and incidence of treatment-emergent AEs will be presented by system organ class and preferred term for each treatment group (number and percentage of participants experiencing at least 1 AE per preferred term as well as the number of observed events per preferred term). Separate tables will be presented by severity and by relationship. All AEs will be presented in full in a comprehensive listing including participant number, treatment regimen, severity, seriousness, action taken, outcome, relationship to treatment, onset/stop, and duration. Details of SAEs and AEs leading to withdrawal will be listed separately. **Adverse Events of Special Interest** AESIs, including liver function abnormalities, ECG/cardiac events, infections, and neutropenia, will be tabulated and summarized by treatment group and overall.
Secondary	**Vital Signs**

Endpoint	Statistical Analysis Methods
	Vital signs will be summarized as observed values and change from baseline by treatment group and overall. **Physical Examination** Physical examination results will be listed by subject ID and body system. **ECG** ECG parameters will be summarized as observed values and change from baseline by treatment group and study visit. Abnormal findings ("normal", "abnormal, not clinically significant", and "abnormal, clinically significant") will be summarized by the number and percentage within each category, and change from baseline will be summarized by shift tables. **Clinical Laboratory** Laboratory parameters will be summarized as observed values and change from baseline by treatment group and study visit. Values outside the normal range will be summarized by the number and percentage within each category, and change from baseline will be summarized by shift tables. **Safety and PK Relationships** The safety correlation analysis with drug concentration may be done for the safety parameters shown to be sensitive to alvelestat exposure. The comparison of exposure will be done between groups with non-clinically significant changes and clinically significant changes in safety parameters using graphic and statistical methods where data allow.
Exploratory	The details will be described in the SAP finalized before database lock.

10.3.3. Other Analyses

PK, PD, and biomarker exploratory analyses will be described in the SAP finalized before database lock.

10.3.4. Interim Analyses

No interim analysis for efficacy is planned for this study. An interim safety analysis will be conducted when 30 subjects have completed the study.

11. References

1. Vestbo J, Hurd SS, Agusti AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65.

2. Centers for Disease C, Prevention. Chronic obstructive pulmonary disease among adults--United States, 2011. MMWR Morb Mortal Wkly Rep 2012;61:938-43.

3. Qaseem A, Wilt TJ, Weinberger SE, et al. Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. Ann Intern Med 2011;155:179-91.

4. Vogelmeier CF, Criner GJ, Martinez FJ, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. Arch Bronconeumol 2017;53:128-49.

5. Barnes PJ. Chronic obstructive pulmonary disease. N Engl J Med 2000;343:269-80.

6. Wells JM, O'Reilly PJ, Szul T, et al. An aberrant leukotriene A4 hydrolase-proline-glycine-proline pathway in the pathogenesis of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2014;190:51-61.

7. Stoller JK, Aboussouan LS. Alpha1-antitrypsin deficiency. Lancet 2005;365:2225-36.

8. Stoller JK, Aboussouan LS. A review of alpha1-antitrypsin deficiency. Am J Respir Crit Care Med 2012;185:246-59.

9. Greulich T. Alpha-1-Antitrypsin Deficiency: Disease Management and Learning from Studies. COPD 2017;14:S8-S11.

10. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med 2009;360:2749-57.

11. Chapman KR, Burdon JG, Piitulainen E, et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. Lancet 2015;386:360-8.

12. Campos MA, Geraghty P, Holt G, et al. The Biological Effects of Double-Dose Alpha-1 Antitrypsin Augmentation Therapy. A Pilot Clinical Trial. Am J Respir Crit Care Med 2019;200:318-26.

13. Cosio MG, Bazzan E, Rigobello C, et al. Alpha-1 Antitrypsin Deficiency: Beyond the Protease/Antiprotease Paradigm. Ann Am Thorac Soc 2016;13 Suppl 4:S305-10.

14. Ferrarotti I, Corsico AG, Stolk J, et al. Advances in Identifying Urine/Serum Biomarkers in Alpha-1 Antitrypsin Deficiency for More Personalized Future Treatment Strategies. COPD 2017;14:56-65.

15. Greene CM, Marciniak SJ, Teckman J, et al. alpha1-Antitrypsin deficiency. Nat Rev Dis Primers 2016;2:16051.

16. Duvoix A, Roussel BD, Lomas DA. Molecular pathogenesis of alpha-1-antitrypsin deficiency. Rev Mal Respir 2014;31:992-1002.

17. Chamba A, Afford SC, Stockley RA, Burnett D. Extracellular proteolysis of fibronectin by neutrophils: characterization and the effects of recombinant cytokines. Am J Respir Cell Mol Biol 1991;4:330-7.

18. Wells JM, Gaggar A, Blalock JE. MMP generated matrikines. Matrix Biol 2015;44-46:122-9.

19. Russell DW, Wells JM, Blalock JE. Disease phenotyping in chronic obstructive pulmonary disease: the neutrophilic endotype. Curr Opin Pulm Med 2016;22:91-9.

20. Luisetti M, Ma S, Iadarola P, et al. Desmosine as a biomarker of elastin degradation in COPD: current status and future directions. Eur Respir J 2008;32:1146-57.

21. Pelham F, Wewers M, Crystal R, Buist AS, Janoff A. Urinary excretion of desmosine (elastin cross-links) in subjects with PiZZ alpha-1-antitrypsin deficiency, a phenotype associated with hereditary predisposition to pulmonary emphysema. Am Rev Respir Dis 1985;132:821-3.

22. Turino GM, Lin YY, He J, Cantor JO, Ma S. Elastin degradation: an effective biomarker in COPD. COPD 2012;9:435-8.

23. Ma S, Turino GM, Lin YY. Quantitation of desmosine and isodesmosine in urine, plasma, and sputum by LC-MS/MS as biomarkers for elastin degradation. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:1893-8.

24. Deslee G, Woods JC, Moore CM, et al. Elastin expression in very severe human COPD. Eur Respir J 2009;34:324-31.

25. Ma S, Lin YY, Turino GM. Measurements of desmosine and isodesmosine by mass spectrometry in COPD. Chest 2007;131:1363-71.

26. Stolk J, Veldhuisen B, Annovazzi L, et al. Short-term variability of biomarkers of proteinase activity in patients with emphysema associated with type Z alpha-1-antitrypsin deficiency. Respir Res 2005;6:47.

27. Bihlet AR, Karsdal MA, Sand JM, et al. Biomarkers of extracellular matrix turnover are associated with emphysema and eosinophilic-bronchitis in COPD. Respir Res 2017;18:22.

28. He J, Turino GM, Lin YY. Characterization of peptide fragments from lung elastin degradation in chronic obstructive pulmonary disease. Exp Lung Res 2010;36:548-57.

29. Senior RM, Griffin GL, Mecham RP, Wrenn DS, Prasad KU, Urry DW. Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. J Cell Biol 1984;99:870-4.

30. Stolz D, Leeming DJ, Kristensen JH, et al. Systemic Biomarkers of Collagen and Elastin Turnover Are Associated With Clinically Relevant Outcomes in COPD. Chest 2017;151:47-59.

31. Ma S, Lin YY, He J, Rouhani FN, Brantly M, Turino GM. Alpha-1 antitrypsin augmentation therapy and biomarkers of elastin degradation. COPD 2013;10:473-81.

32. Stevens T, Ekholm K, Granse M, et al. AZD9668: pharmacological characterization of a novel oral inhibitor of neutrophil elastase. J Pharmacol Exp Ther 2011;339:313-20.

33. Vogelmeier C, Aquino TO, O'Brien CD, Perrett J, Gunawardena KA. A randomised, placebo-controlled, dose-finding study of AZD9668, an oral inhibitor of neutrophil elastase, in patients with chronic obstructive pulmonary disease treated with tiotropium. COPD 2012;9:111-20.

34. Kuna P, Jenkins M, O'Brien CD, Fahy WA. AZD9668, a neutrophil elastase inhibitor, plus ongoing budesonide/formoterol in patients with COPD. Respir Med 2012;106:531-9.

35. Elborn JS, Perrett J, Forsman-Semb K, Marks-Konczalik J, Gunawardena K, Entwistle N. Efficacy, safety and effect on biomarkers of AZD9668 in cystic fibrosis. Eur Respir J 2012;40:969-76.

36. Gunawardena KA, Gullstrand H, Perrett J. Pharmacokinetics and safety of AZD9668, an oral neutrophil elastase inhibitor, in healthy volunteers and patients with COPD. Int J Clin Pharmacol Ther 2013;51:288-304.

37. Stockley R, De Soyza A, Gunawardena K, et al. Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis. Respir Med 2013;107:524-33.

38. Nordenmark LH, Taylor R, Jorup C. Feasibility of Computed Tomography in a Multicenter COPD Trial: A Study of the Effect of AZD9668 on Structural Airway Changes. Adv Ther 2015;32:548-66.

39. Woodruff PG, Agusti A, Roche N, Singh D, Martinez FJ. Current concepts in targeting chronic obstructive pulmonary disease pharmacotherapy: making progress towards personalised management. Lancet 2015;385:1789-98.

40. Ma S, Lin YY, Cantor JO, et al. The Effect of Alpha-1 Proteinase Inhibitor on Biomarkers of Elastin Degradation in Alpha-1 Antitrypsin Deficiency: An Analysis of the RAPID/RAPID Extension Trials. Chronic Obstr Pulm Dis 2016;4:34-44.

41. Nelson DR, Teckman J, Di Bisceglie AM, Brenner DA. Diagnosis and management of patients with alpha1-antitrypsin (A1AT) deficiency. Clin Gastroenterol Hepatol 2012;10:575-80.

42. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.

43. Meguro M, Barley EA, Spencer S, Jones PW. Development and Validation of an Improved, COPD-Specific Version of the St. George Respiratory Questionnaire. Chest 2007;132:456-63.

44. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J 2009;34:648-54.

45. Comstock GW, Tockman MS, Helsing KJ, Hennesy KM. Standardized respiratory questionnaires: comparison of the old with the new. Am Rev Respir Dis 1979;119:45-53.

46. Eakin EG, Resnikoff PM, Prewitt LM, Ries AL, Kaplan RM. Validation of a new dyspnea measure: the UCSD Shortness of Breath Questionnaire. University of California, San Diego. Chest 1998;113:619-24.

47. Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. Eur Radiol 2010;20:2707-14.

12. Appendices

Appendix 1: Abbreviations, Trademarks & Glossary

Abbreviation	Description
AAT	Alpha-1antitrypsin
AATD	Alpha-1 antitrypsin deficiency
Aα-Val360	Fragment from neutrophil elastase cleavage of elastin at Aα-Val360 site
AcPGP	Acetylated Proline-Glycine-Proline
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the curve
BAL	Bronchoalveolar lavage
bid	Twice (2 times) a day
C1M	MMP-derived collagen type I breakdown fragment
C6M	MMP-derived collagen type VI breakdown fragment
CAT	COPD assessment test
Cat g	Cathepsin G
CF	Cystic fibrosis
CFR	United States Code of Federal Regulations
CMP	Complete metabolic profile
CMV	Cytomegalovirus
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CRO	Clinical Research Organization
CT	Computerized or computed tomography

CYP2C9	Cytochrome P450 2C9
DCC	Data Coordinating Center
DES	Desmosine
dL	Decilitre
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
ECG	Electrocardiogram
eCRF	Electronic case report form
EL-CG	Elastin degradation mediated by Cathepsin G
EL-NE	Elastin degradation mediated by NE
EL-P3	Elastin like polypeptide 3
EOT	End of treatment
EP-3	Elastin peptide 3
FEV1	Forced expiratory volume in 1 second
FEV1/FVC	Forced expiratory volume in 1 second divided by the forced vital capacity
FVC	Forced vital capacity
FIB-4	Fibrosis-4
FU	Follow-up
FVC	Forced vital capacity
g	Gram
GCP	Good Clinical Practice
h	Hour
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
hsCRP	High-sensitivity C-reactive protein
HRT	Hormone replacement therapy

HSV	Herpes simplex virus
Hy's Law	a rule of thumb that a patient is at high risk of a fatal drug-induced liver injury (DILI) if given a medication that causes hepatocellular injury (not cholestatic injury) with jaundice
IC50	the concentration of an inhibitor where the response (or binding) is reduced by half
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-1β	Interleukin 1 beta
IL-6	interleukin-6
IL-8	interleukin-8
IND	Investigational new drug
INR	International normalized ratio
IRB	Institutional Review Board
IWRS	Interactive web response system
Ki	Inhibitory constant
kPa	kiloPascal, a unit of force
LSM	Liver stiffness measurement
LTB4	Leukotriene b4
MedDRA	Medical Dictionary for Regulatory Activities
mGy	milliGray, a unit of radiation
mg/dL	Milligrams per decilitre
mIU	Milli International Units
MMP	Matrix metalloproteinase
MMRC	Modified medical research council
MOP	Methods of procedures
MPO	Myeloperoxidase

MS	Millisecond
mSV	milliSever
NCATS	National Center for Advancing Translational Science
NCT	National Clinical Trial
NE	Neutrophil elastase
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NSAID	Non-steroidal anti-inflammatory drug
Pa	Pascal
PD	Pharmacodynamics
PI	Principal investigator
Pi*ZZ	Alpha-1 ZZ genotype
Pi*SZ	Alpha-1 SZ genotype
Pi*Null, Null	Alpha-1 Null genotype
PGP	P-glycoprotein
PK	Pharmacokinetics
PR	time from the onset of the P wave to the start of the QRS complex on ECG
PR3	Proteinase 3
PROs	Patient reported outcomes
PRO-C6	Released C-terminal pro-peptide of type VI collagen
QRS	Represents ventricular depolarization on ECG
QT	The duration of ventricular depolarization and repolarization on ECG
QTc	Corrected QT interval
QTcF	QTc by Fridericia's correction method
QTcV	QTc by Van de Water's correction formula
RANTES	Regulated on activation, normal T cell expressed and secreted

RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical analysis software
SGRQ-C	St. George's Respiratory Questionnaire compact version
SoA	Schedule of Activities
SOB	Shortness of breath
SOP	Standard operating procedure
SOBQ	Shortness of breath questionnaire
Sp02	Pulse oximetry
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Total bilirubin
ug	Microgram
ULN	Upper limit of normal
uM	Micromolar
UPs	Unanticipated problems
VZV	Varicella zoster virus
WBC	White blood cell
Wk.	Week

Appendix 2: Clinical Laboratory Tests

- The tests detailed in the table below will be performed by the local laboratories.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6.1 or Section 6.2, respectively.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit	MCV MCH %Reticulocytes	WBC count with differential (% and absolute counts): Neutrophils Lymphocytes Monocytes Eosinophils Basophils	Prothrombin Time (PT) Partial prothromboplastin time (PTT) International normalized ratio (INR)
Clinical Chemistry[1]	Blood urea nitrogen	Potassium	AST/Serum glutamic-oxaloacetic transaminase (SGOT)	Total and direct bilirubin
	Creatinine and estimated glomerular filtration rate clearance	Sodium	ALT/Serum glutamic-pyruvic transaminase (SGPT)	Total protein
	Alkaline phosphatase	Calcium		
Other Screening Tests	• Urine pregnancy test during the study (as needed for women of childbearing potential) • Serum human chorionic gonadotropin pregnancy test (as needed for women of childbearing potential) • Alpha-1 antitrypsin blood protein levels • Serology (HIV antibody, hepatitis B surface antigen and hepatitis B antibody, and hepatitis C virus antibody) • All study-required laboratory assessments will be performed at the local laboratory, with the exceptions of the samples (blood, sputum, BAL) that will be sent to a central laboratory for curation and analysis. • The results of each test must be entered into the CRF as guided by the CRF Completion Guidelines.			

[1] Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 8.1. All events of ALT ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and INR >1.5, if INR is measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE. [2] Local urine testing will be standard.

Investigators must document their review of each laboratory safety report.

Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines.
  - Applicable laws and regulations under the IND.

- The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB by the Investigator and reviewed and approved by the IRB & Competent Authority/Regulatory Authority before the study is initiated.

- Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
  - Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures.
  - Overall conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, and all other applicable local regulations.

Financial Disclosure

Principal Investigators and Site Investigators will provide the DCC and the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Informed Consent Process

- The Site Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB or study site.

- The source documentation and CRF must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

- A copy of the ICF(s) must be provided to the participant or his/her legally authorized representative.

Data Management

- The study will use an electronic data entry system (eDES), a web-based platform used for data entry, curation, archiving, and notification. The eDES was developed and is managed by the DCC.
- CRFs can be printed from the eCRF generated by and available through the eDES. The Site Investigator / Site PI will be responsible for data entry into the system. The DCC will monitor for data quality and will query sites as needed.
- Oversight for the study will be done via risk-based monitoring.
- The DCC will centrally monitor sites can provide on-site monitoring if needed for any high risk clinical site (e.g. those with data anomalies or a higher frequency of errors, protocol violations, or dropouts relative to other site).
- If adverse events require hospitalization, study participants will be cared for by the hospital staff at the local sites as per local policy. The study team will monitor the status of the patient while hospitalized, but will not be required to provide all aspects of care for these individuals

Data Protection

- Participants will be assigned a unique identifier by the DCC. Any participant records or datasets that are transferred to the DCC will contain the identifier only; participant names or any information that would make the participant identifiable will not be transferred.

- The participant must be informed that his/her personal study-related data will be used by the DCC in accordance with local data protection law. The level of disclosure must also be explained to the participant.

- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the DCC, Mereo BioPharma, by appropriate IRB members, and by inspectors from regulatory authorities.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the DCC or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

- The Sponsor or designee is responsible for the data management of this study, including quality checking of the data.

- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the Investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the NIH. No records may be transferred to another location or party without written notification to the NIH and the DCC.

Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

- Data reported on the CRF or entered in the electronic CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

- Definition of what constitutes source data can be found in the Study Manual and Monitoring Plan.

Study and Site Closure

The NIH, Mereo, UAB, the FDA, the IRB, or a designee may stop the study or study site participation in the study for medical, safety, regulatory, administrative, or other reasons consistent with applicable laws, regulations, and GCP.

Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

AE Definition
• An AE is any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE/SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE/SAE if they fulfil the definition of an AE/SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Unanticipated Problem (UP) Definition
• In general, an AE observed during the conduct of a study should be considered an **Unanticipated Problem (UP)** involving risk to human subjects, and reported to the IRB, ***only*** if it were unexpected, serious, and would have implications for the conduct of the study (e.g., requiring a significant, and usually safety-related, change in the protocol such as revising inclusion/exclusion criteria or including a new monitoring requirement, informed consent, or investigator's brochure). • An individual AE occurrence ***ordinarily*** does not meet these criteria because, as an isolated event, its implications for the study cannot be understood.

- AEs listed in the Investigator's Brochure (IB) are not by definition UPs because they have been previously observed with a drug, and would not be considered unexpected and thus would not be unanticipated problems.
- Possible exceptions would include situations in which the specificity or severity of the event is not consistent with the description in the investigator's brochure, or it can be determined that the observed rate of occurrence for a serious, expected AE in the clinical trial represents a clinically important increase in the expected rate of occurrence

Events Meeting UP Definition
• A single occurrence of a serious, unexpected event that is uncommon and strongly associated with drug exposure (such as angioedema, agranulocytosis, hepatic injury, or Stevens-Johnson syndrome). • A single occurrence, or more often a small number of occurrences, of a serious, unexpected event that is not commonly associated with drug exposure, but uncommon in the study population (e.g., tendon rupture, progressive multifocal leukoencephalopathy). • Multiple occurrences of an AE that, based on an aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects (e.g., a comparison of rates across treatment groups reveals higher rate in the drug treatment arm versus a control). • An AE that is described or addressed in the investigator's brochure, protocol, or informed consent documents, but occurs at a specificity or severity that is inconsistent with prior observations. For example, if transaminase elevation is listed in the investigator's brochure and hepatic necrosis is observed in study subjects, hepatic necrosis would be considered an unanticipated problem involving risk to human subjects. • A serious AE that is described or addressed in the investigator's brochure, protocol, or informed consent documents, but for which the rate of occurrence in the study represents a clinically significant increase in the expected rate of occurrence (ordinarily, reporting would only be triggered if there were a credible baseline rate for comparison). • Any other AE or safety finding (e.g., based on animal or epidemiologic data) that would cause the sponsor to modify the investigator's brochure, study protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death or;
b. Is life-threatening or; The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization or; In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity or;

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect or;

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording AEs and SAEs

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to the DCC in lieu of completion of the AESI/AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by the DCC. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to the DCC.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

  An event is defined as "serious" when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the DCC.**
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the DCC to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide the DCC with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to the DCC within 24 hours of receipt of the information.

SAE Reporting to DCC via eCRF

- SAEs will be reported to the DCC via the eCRF.
- Initial reporting can be updated when additional information is obtained but this should not delay submission.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

- An adverse reaction (AR) is any untoward or unfavorable medical occurrence in a clinical research study participant, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.
- An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.
- SUSAR events are AR events that fulfil criteria for seriousness (outlined in SAE above). SUSARs should be reported to the DCC, NIH/NCATS, Mereo, and the FDA using the instructions listed for SAE reporting.

Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Alvelestat (MPH966) has a low genotoxic potential and reproductive toxicology studies have not demonstrated any effects in either the reproductive function or in embryo-fetal development. Therefore, the following precautions are required to protect women of childbearing potential and female partners of males in the study.

Definitions

Woman of Childbearing Potential

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

Women in the following categories are not considered women of childbearing potential

1. Premenopausal female with one of the following:
   a) Documented hysterectomy
   b) Documented bilateral salpingectomy
   c) Documented bilateral oophorectomy

   Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

2. Premenarchal

3. Postmenopausal female
   a) A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single follicle stimulating hormone measurement is insufficient.
   b) Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

Contraception Guidance

Male participants

Male participants with female partners of childbearing potential are eligible to participate if they agree to the following:

1. Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent for duration of study and for 4 days from last dose
2. Female partner is using a highly effective contraceptive method
3. Agree to use a male condom plus an additional method with a failure rate of <1% per year as described in the table below when having penile-vaginal intercourse with a woman of childbearing potential.
4. Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration and for 4 days from last dose.
5. Refrain from donating sperm for duration of study and for 4 days from last dose.

Highly Effective Contraceptive Methods That Are User Dependent *Failure rate of <1% per year when used consistently and correctly[a].*
Combined (estrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation[b] • Oral • Injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.)*
Sexual abstinence *(Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)*
NOTES: a) Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies. b) Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 4 days from last dose.

Female participants

Female participants of reproductive potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in the table above.

Pregnancy Testing

Women of childbearing potential should only be included after a confirmed menstrual period and a negative highly sensitive serum pregnancy test.

Additional pregnancy testing should be performed at regular intervals as per the SoA during the treatment period and at 28 days after the last dose of study treatment.

Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

Pregnancy testing, with a sensitivity of 5 mIU/mL will be performed and assayed at the study site.

Collection of Pregnancy Information

Male participants with partners of reproductive potential who become pregnant

Investigator will attempt to collect pregnancy information on any female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.

If a pregnancy is reported for a subject's partner, study treatment will be immediately discontinued.

After obtaining the necessary signed ICF from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the DCC within 24 hours of learning of the partner's pregnancy.

Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the DCC.

Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

Female participants who become pregnant

Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study.

Information will be recorded on the appropriate form and submitted to the DCC within 24 hours of learning of a participant's pregnancy.

Participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on participant and neonate, which will be forwarded to the DCC. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.

Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
A spontaneous abortion is always considered to be an SAE and will be reported as such.

Any SAE occurring as a result of a post-study pregnancy that is considered reasonably related to the study treatment by the Investigator will be reported to the DCC as described in Appendix 4. While the Investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating will be withdrawn from the study.

Appendix 6: Study Organization

UAB	Name	Title	Contact
Study PI, Medical Oversight	Mark Dransfield, MD	Professor	205-996-0101 mdransfield@uabmc.edu
Study PI, DCC Oversight	Chichi Aban, PhD	Professor	205-934-2782 caban@uab.edu
Sub-Investigator	Mike Wells, MD	Associate Professor	205-996-0101 jmwells@uabmc.edu
Sub-Investigator	Gary Cutter, PhD	Professor	205-975-5048 cutterg@uab.edu
Financial and Subcontracting Oversight	Casondra Matthews	Clinical Research Administrator III	205-934-5555 casondramatthews@uabmc.edu
Regulatory Oversight	Elizabeth Westfall MPH	Director-Clinical Research Administration	205-934-9282 ewestfall@uabmc.edu
NIH/NCATS			
NCATS Oversight	Bobbie Ann Austin, PhD	Program Officer	Ph: 301.435.0824 bobbieann.mount@nih.gov
NHLBI Oversight and Medical Monitor	Antonello (Tony) Punturieri, MD, PhD	Program Director, DLD at NHLBI	Ph: (301) 592-8573 punturieria@nhlbi.nih.gov
Mereo BioPharma			
Mereo BioPharma Medical Expert and Sponsor Signatory	Jackie Parkin	Therapeutic Area Head	Mereo BioPharma 4 Ltd. 1 Cavendish Place London, W1G 0QF
Mereo BioPharma Clinical Operations Oversight	William Moore	Clinical Operations Lead	
Participating Centers			
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University of Utah	Cheryl Pirozzi, MD	Site PI	Cheryl.Pirozzi@hsc.utah.edu
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Data Safety Monitoring Board			
Brendan McGuire, MD	Gastroenterologist, UAB	bmcguire@uabmc.edu	
Robert Reed, MD	Pulmonologist, University of Maryland	rreed@som.umaryland.edu	
Amber Salter, PhD	Statistician, Washington University	amber@wustl.edu	

Appendix 7: Summary of Versions and Changes

Protocol	Version	Date	Summary of Change
Original	1.0	September 5, 2018	Not Applicable
Revision 1	1.1	October 26, 2018	Eligibility and safety modifications per FDA request (see also Table of Protocol changes October 26, 2018)
Revision 2	1.2	July 31, 2019	Eligibility and safety modifications (see also Table of Protocol changes July 31, 2019)
Revision 3	1.3	February 18, 2020	Eligibility modifications to include participants on augmentation therapy and safety updates (also see Table of Protocol changes February 18, 2020
Revision 4	1.4	February 10, 2021	Eligibility modifications to include participants with other rare genotypes/phenotypes at increased risk for emphysema. (also see Table of Protocol changes February 16, 2021)